

# THE HIGHLY UNSATURATED ACIDS IN SARDINE OIL.

## XVII. RE-EXAMINATION OF THE STRUCTURE OF EICOSATETRAENOIC ACID

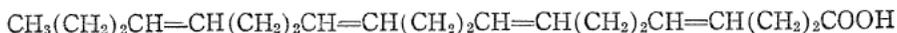
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In the 5th report of this series,<sup>1)</sup> eicosatetraenoic acid was separated from sardine oil, and its amyl ester was subjected to ozonolysis. Results of an examination of the ozonolysis product indicated a 4, 8, 12, 16-tetraenoic structure. Later, eicosatetraenoic acid in bonito oil was found to have the same structure as the acid of sardine oil by Matsuda.<sup>2)</sup> On the other hand, eicosatetraenoic acid, named arachidonic acid, has been known for a long time as a fatty acid component of lipids from the organs of domestic animals. The structure of arachidonic acid from beef suprarenal lipids was first studied in 1940 by Shinowara and Brown<sup>3)</sup> who assigned a 6, 10, 14, 18-tetraenoic structure to it. In the same year, however, Dolby, Nunn and Smedley-Maclean<sup>4)</sup> found that arachidonic acid has a 5, 8, 11, 14-tetraenoic structure which was also confirmed by later investigations of Mowry, Brode and Brown<sup>5)</sup> and Arcus and Smedley-Maclean.<sup>6)</sup> Although the literatures cited above seem to indicate that eicosatetraenoic acid of marine animal oils differ in its structure from arachidonic acid of lipids from the organs of terrestrial animal oils, some authors<sup>7)</sup> appear not to regard the 4, 8, 12, 16-tetraenoic structure of the former acid as conclusive. Hence, a re-examination of the structure of eicosatetraenoic acid of sardine oil was undertaken by the present authors.

Eicosatetraenoic acid was separated from sardine oil, and its ethyl ester was oxidized with potassium permanganate in acetone. Among the oxidative scission products, ethyl hydrogen succinate, succinic acid and butyric acid were identified. It follows from these results that ethyl eicosatetraenoate has the terminal groups  $\text{CH}_3(\text{CH}_2)_2\text{CH}=\text{}$  and  $=\text{CH}(\text{CH}_2)_2\text{COOC}_2\text{H}_5$  and three of the intermediate group  $=\text{CH}(\text{CH}_2)_2\text{CH}=\text{}$ . Accordingly, the structure of the free acid is expressed by the following formula:



Thus the 4, 8, 12, 16-tetraenoic structure of this acid, as reported previously, is reconfirmed.

### Experimental

#### 1. Separation of Eicosatetraenoic Acid from Sardine Oil

A highly unsaturated acid concentrate was prepared from winterized sardine oil by the sodium salt acetone method, and its methyl esters were fractionally

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distilled. A portion (203 g) of the higher methyl ester fraction (S.V. 177.8 and I.V. 365.1, described in the 15th report of this series<sup>5)</sup>) was subjected to a further fractional distillation, yielding 36.1 g of a fraction of B.P. 200°-205°C/1 mm, S.V. 177.2 and I.V. 355.2. Fatty acids obtained by saponification of this fraction were fractionally precipitated as sodium salts from acetone as shown in Table 1.

TABLE 1  
Fatty acids, 33.2 g, N.V. 185.1, I.V. 370.4

F.A. from 1st ppt. 2.0 g N.V. 184.0 I.V. 270.6	F.A. from 2nd ppt. 12.1 g N.V. 184.2 I.V. 350.2	F.A. from filtrate 18.5 g N.V. 185.5 I.V. 390.5
F.A. from 1st ppt. 7.6 g N.V. 184.0 I.V. 335.2	F.A. from 2nd ppt. 2.5 g N.V. 184.4 I.V. 372.5	F.A. from filtrate 1.5 g N.V. 184.6 I.V. 392.0
F.A. from ppt. (a) 5.5 g N.V. 183.9 I.V. 334.1	F.A. from filtrate (b) 2.0 g N.V. 184.4 I.V. 336.1	

The fatty acid (a) in Table 1 showed neutralization and iodine values which are close to the calculated values for eicosatetraenoic acid (N.V. 184.3 and I.V. 333.5). It had  $d_4^{20}$  0.9266,  $n_D^{20}$  1.4916 and Mol. Refr. 95.26 (Calc. for  $C_{20}H_{32}O_2$ , 94.23). The fatty acid (b) gave an ether insoluble bromide which had Br content 66.56% (Calc. for  $C_{20}H_{32}O_2Br_2$ , 67.74%) and turned black at about 240° C. Hydrogenated product of the fatty acid (b) showed, after recrystallization from ethanol, M.P. 75° C and N.V. 178.9 and was identified with arachidic acid (M.P. 75° C and N.V. Calc., 179.5).

## 2. Oxidation of Ethyl Eicosatetraenoate with Potassium Permanganate in Acetone

Eicosatetraenoic acid (the fatty acid a in Table 1) was converted into ethyl ester (5.2 g). This was dissolved in 200 cc of acetone, 70 g of powdered potassium permanganate was added to the solution in small portions, and the mixture was refluxed for 15 hours in order to complete oxidation. After distilling off acetone, 150 cc of water was added to the residue, and sulfur dioxide was passed into the mixture in order to reduce the excess of potassium permanganate. The mixture was then filtered through a wet filter paper. Oily substances (A), together with manganese oxides on the filter paper, were washed with hot water. Filtrate and washings were combined, neutralized with sodium carbonate, evaporated to about 30 cc volume, acidified with sulfuric acid, and then extracted twice with 500 cc of ether. The ether solution was washed with a saturated solution of sodium chloride. After dehydration and distillation of the ether solution, there was obtained a residue which consisted of a mixture of solid and liquid. The residue was separated into petroleum ether insoluble portion (B) and petroleum ether soluble portion (C) by washing twice with 20 cc of petroleum ether.

*Oily substances (A).* Oily substances (A), together with manganese oxides

obtained above, were extracted three times with 200 cc of ether, and the ether solution was washed with a solution of sodium carbonate, by which the acidic substances entered into the aqueous solution as sodium salts. The aqueous solution was separated, acidified with sulfuric acid, and extracted with a relatively large amount of ether. The ether solution, after washing with a little water and dehydration, was distilled, and there was obtained 1.1 g of a liquid substance which had N.V. 379.6 and was considered to be ethyl hydrogen succinate (N.V. Calc., 383.9). Saponification of this substance gave a free acid, which had M.P. 179°-180° C and N.V. 949.1 after recrystallization from ethanol-petroleum ether, and showed no depression of melting point when mixed with succinic acid (M.P. 182°-183° C and N.V. Calc., 950.3).

*Petroleum ether insoluble portion (B).* This portion (1.5 g) was washed with a little cold ether and recrystallized twice from ethanol-petroleum ether, yielding succinic acid of M.P. 180°-181° C and N.V. 948.6.

*Petroleum ether soluble portion (C).* This portion (0.5 g) was still contaminated with a small amount of solid. The latter was removed by further treatment with petroleum ether. Since the liquid portion thus obtained was suspected to be contaminated with non-acidic substances, it was dissolved in an excess of sodium carbonate solution, and non-acidic substances were removed by extraction with ether. The carbonate solution was acidified with sulfuric acid, and the liberated acidic substances were taken up with a relatively large amount of ether. The ether solution, after washing with a saturated solution of sodium chloride and dehydration, was distilled, and there was obtained a residue which had an odor of butyric acid and showed N.V. 625.9 (Calc. for butyric acid, 636.8). Hydroxamic acid was prepared from this residue, and its ethanol solution was subjected to paper chromatographic analysis with the results shown in Table 2, from which the liquid portion was found to consist mainly of butyric acid.

TABLE 2

	<i>R<sub>f</sub></i> (Ascending method)	Coloration
Hydroxamic acid from the liquid portion	0.57	Purple
Butyro-hydroxamic acid	0.58	Purple

Filter paper: "Toyo Filter Paper No. 2"; developer: ethyl acetate; temperature: 17°-18° C; chromogenic reagent: 10% solution of ferric chloride in ethanol.

### Summary

Eicosatetraenoic acid was separated from sardine oil, and its ethyl ester was oxidized with potassium permanganate in acetone. Among the oxidative scission products, butyric acid, succinic acid and ethyl hydrogen succinate were identified. Hence the 4,8,12,16-tetraenoic structure of this acid, as shown in the 5th report of this series, was reconfirmed.

### References

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